

EXHIBIT J

Toward Personalized Treatment of Advanced Biliary Tract Cancers

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Abstract: Biliary tract cancers (BTC) are a relatively rare heterogeneous group of four to five anatomically distinct cancers whose prognosis is poor, even in the setting of attempted curative resection. Curative resection, in itself, is much less common than locally advanced unresectable and/or overt metastatic disease at presentation. Standard chemotherapy options are generally palliative for advanced BTC (aBTC), and recently the combination of gemcitabine with cisplatin has emerged as the standard-of-care providing a median overall survival of approximately one year. A movement toward molecularly based personalized cancer therapy has occurred in recent years, including for aBTC, with a number of pathways emerging as putative therapeutic targets. This review will briefly summarize the epidemiology, etiology, and general prognosis of BTC, then discuss the data supporting current standard cytotoxic treatments of aBTC, and proceed to focus on the molecular features of this heterogeneous set of diseases. Finally, we review strategies which will potentially improve our ability to individualize therapy and, ultimately, clinical outcomes in the future. [Discovery Medicine 13(74):41-57, July 2012]

Introduction

Biliary tract cancers (BTC) are comprised of four distinct adenocarcinomas: a) gallbladder carcinoma (GBC); b) intrahepatic cholangiocarcinoma (IHCC); c)

hilar cholangiocarcinoma (HCC), also known as a Klatskin tumor and further sub-classified by the Bismuth criteria (Bismuth *et al.*, 1992); and d) extrahepatic cholangiocarcinoma (EHCC) (Figure 1). Confusingly, ampullary carcinoma has occasionally been included and other times not included in the definition of EHCC; however, we consider this a separate tumor type that is not within the scope of this review, and will not be discussed hereafter. The latter three tumor types -- IHCC, HCC, and EHCC -- have often been grouped together under the general term cholangiocarcinoma (CC), yet the 7th Edition of 2010 AJCC (American Joint Committee on Cancer) staging system classifies each of the four types as separate entities with distinct staging and biological properties (Edge *et al.*, 2010). Cure is only attainable with surgery. However, in general, due to the advanced stage at presentation and the aggressive nature of these tumors, only 10-20% of patients are ever deemed surgical candidates (Hezel and Zhu, 2008). Even after resection, the rate of recurrence is approximately 60% for BTC as a whole and, accordingly, the 5-year overall survival (OS) rates are approximately 5-10% for GBC and 10-40% for CC (de Groen *et al.*, 1999; Furuse *et al.*, 2012). Another series reported a 5-year OS for those with resected IHCC from 15-40%, for HCC from 10-40%, and for EHCC from 23-50% (Aljifry *et al.*, 2009). A recent retrospective analysis of 1,057 patients over 24 years reported that definitive surgery was performed in 41% of patients, and adjuvant chemotherapy (CT) or chemotherapy with radiation (CRT) given in 20% and 8%, respectively; CT or CRT was given for unresectable or metastatic disease in 42% of the cases as the first-line therapy. For the entire cohort the median OS (mOS) was 19.3 months, of which stage IV disease was seen in 63% of GBC, 30% of EHCC, 52% of HCC, and 59% of IHCC at time of original diagnosis (McNamara *et al.*, 2012). Earlier detection of EHCC, as compared to the other BTC subsets, is likely due to earlier heralding biliary obstructive signs and symptoms. The mOS for all stages of IHCC treated with only best supportive care (BSC) has recent-

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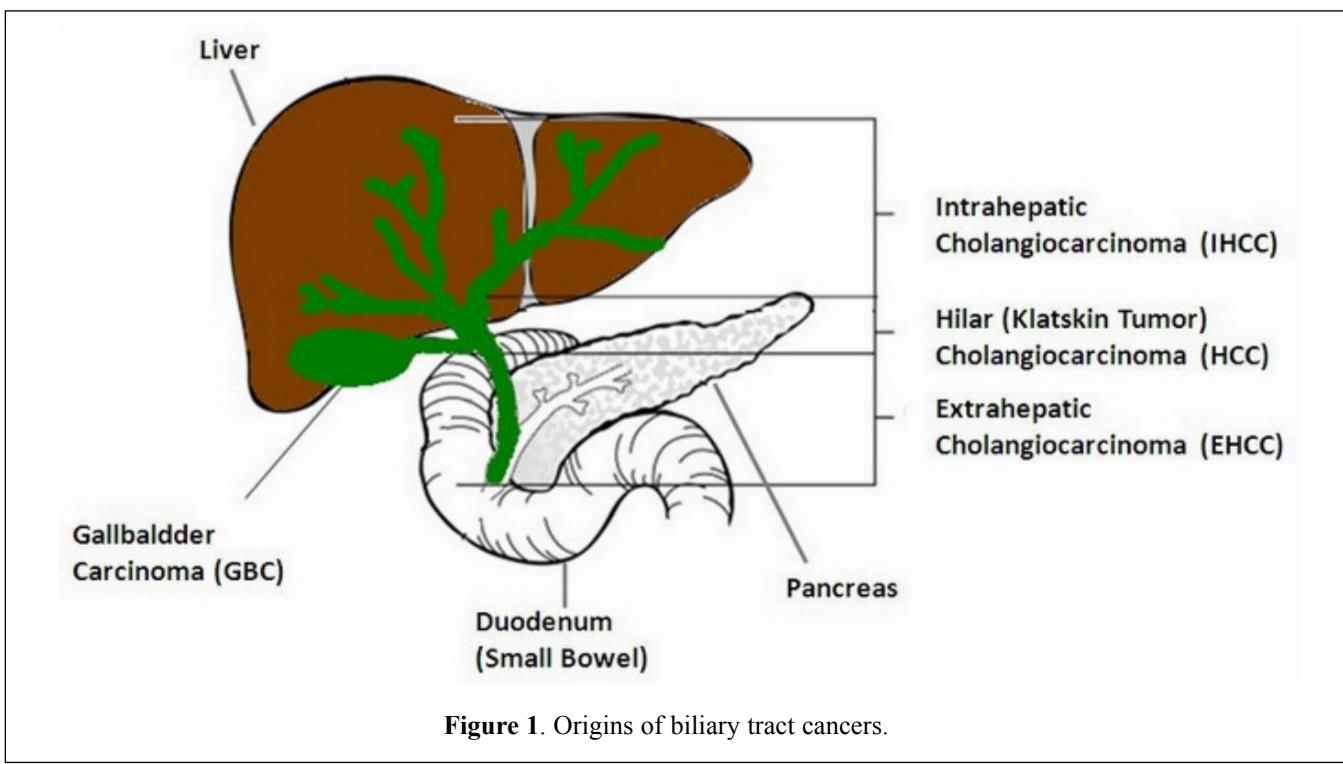
ly been reported in one series to be 3 months, and 5.9 months for EHCC (Park *et al.*, 2009). GBC is considered the most lethal of all four BTC with only 10% presenting as surgical candidates (Zhu *et al.*, 2010a). For the most part, then, we are faced with locally advanced unresectable, *de novo* metastatic, or recurrent disease, for which palliative systemic therapy is indicated.

The introduction of targeted biologic therapies for various malignancies over the last decade has been encouraging, initially for gastrointestinal stromal tumors and chronic myelogenous leukemias, and subsequently for several others including lung, colon, prostate, renal, gastroesophageal, and breast cancers. However, in an orphan disease such as BTC, where the progress has lagged behind the treatment advances of these more common malignancies, there is a sense for renewed hope for the future. Underlying this hope is the premise that malignancies are dependent on certain genetic and epigenetic aberrations of which targeted inhibition with either antibodies and/or small-molecules will lead to significant progress. In this review we will discuss the current landscape and future directions of the treatment of advanced BTC (aBTC), defined here as metastatic or unresectable disease not amenable or refractory to any local therapy. Other clinical settings, including surgery (curative resection or transplant) or locally ablative therapies (radiation, transarterial chemoembolization, radiofrequency ablation, etc.), are beyond the scope of this review; therefore, trials discussed herein, unless

otherwise specified, examined this aBTC patient population as a single group.

Epidemiology and Etiology of Biliary Tract Cancers

BTC account for approximately 3% of all gastrointestinal malignancies. In 2012 in the United States, 9,810 new cases and 3,200 deaths are expected from BTC, excluding IHCC which remains combined with hepatocellular carcinoma in epidemiologic reports (Siegel *et al.*, 2012). Recently, an entity of combined hepatocellular-cholangiocarcinoma has been recognized that may be explained by a common liver progenitor cell and appears to lead to worse surgical outcomes (Ariizumi *et al.*, 2012; Yeh, 2010). For CC, approximately 5-10% are IHCC, 60-70% HCC, and 20-30% EHCC (Aljifry *et al.*, 2009). BTC present in patients aged greater than 60 in approximately 65% of cases. The incidence of BTC varies greatly around the world with much higher rates in Asia compared to Western Europe and the U.S. For example, rates of IHCC have been reported in up to 38-96/100,000 people in Thailand and 0.1-0.2/100,000 in Australia (Shaib and El-Serag, 2004). In the U.S., the incidence of IHCC is observed to be rising for unclear reasons (Charbel and Al-Kawas, 2011). Whereas the majority of patients have no identifiable etiology, known risk factors include chronic inflammatory diseases including primary sclerosing cholangitis, hepatolithiasis, viral hepatitis, HIV, and cirrhosis; parasitic infections, namely liver fluke infection; congenital



defects including choledochal cysts, Caroli's disease, and congenital hepatic fibrosis; chemicals such as dioxin in nitrosamines and asbestos; medications such as oral contraceptive pills and isoniazid; biliary-enteric anastomosis; and other general exposures and behaviors including smoking, obesity, and diabetes. Many of these presumably lead to a state of chronic inflammation, cancer initiation, and progression.

Current Cytotoxic Treatment of Advanced Biliary Tract Cancers

Although well over 100 chemotherapy-based clinical trials for aBTC have been conducted, most have been single arm small trials, only a handful have been randomized, and all treat the four distinct types of BTC as a single entity (Table 1). Surely, this grouping of BTC and less-than-ideal clinical investigation is due to the rarity of the disease. A 1996 trial of 90 patients with advanced pancreatic and biliary cancers of whom 37 had BTC showed an OS benefit to FELV [5-fluorouracil (5-FU), etoposide and leucovorin] chemotherapy over best supportive care (BSC) (6.0 vs. 2.5 months) and reportedly improved quality of life (Glimelius *et al.*, 1996). More recently, the utility of chemotherapy with gemcitabine and oxaliplatin (GEMOX) vs. 5-FU or BSC in GBC patients was reported (Sharma *et al.*, 2010). Eighty one patients were randomized to one of three arms (BSC=27; 5-FU=28; GEMOX=26) with a median progression-free survival (mPFS) of 2.8, 3.5, and 8.5 months in the BSC, 5-FU, and GEMOX arms, respectively ($p<0.001$), and mOS of 4.5, 4.6, and 9.5 months in the BSC, 5-FU, and GEMOX arms, respectively ($p=0.39$). A randomized trial evaluated mitomycin with capecitabine or gemcitabine showing that the mitomycin with capecitabine combination was superior with an OS of 9.25 months (Kornek *et al.*, 2004). Another trial evaluated 5-FU with or without cisplatin showing an improved overall response rate (7.1% vs. 19%) and OS (5 vs. 8 months) in favor of the combination arm, albeit with more toxicity (Ducreux *et al.*, 2005). A trial of 54 patients randomized to FELV vs. ECF (epirubicin, cisplatin, and 5-FU) showed a similar mOS (ECF 9.02 months and FELV 12.03 months; $p=0.2$) and objective response rate (RR; ECF 19.2% and FELV 15%; $p=0.72$) (Rao *et al.*, 2005).

Several other regimens and single agents have been studied (Hezel and Zhu, 2008). Notable representative examples (Table 1) include single agent 5-FU (Choi *et al.*, 2000), 5-FU with cisplatin (Kobayashi *et al.*, 2006), 5-FU with oxaliplatin (Lee *et al.*), capecitabine and oxaliplatin (Nehls *et al.*, 2008), capecitabine and cisplatin (Hong *et al.*, 2007; Kim *et al.*, 2003), single agent gemcitabine (Gebbia *et al.*, 2001; Penz *et al.*, 2001),

gemcitabine with oxaliplatin (Andre *et al.*, 2004; Harder *et al.*, 2006; Jang *et al.*, 2010), gemcitabine and 5-FU (Alberts *et al.*, 2005), irinotecan with 5-FU or gemcitabine (Bhargava *et al.*, 2003; Feisthammel *et al.*, 2007), gemcitabine with capecitabine (Knox *et al.*, 2005; Riechelmann *et al.*, 2007), and gemcitabine with S-1 (Ueno *et al.*, 2012). In general, RR [partial response (PR) + complete response (CR)] varied between 10-30% with mOS of approximately 6-14 months. In general, a longer mOS was seen with combination cytotoxic regimens.

A large meta-analysis of 104 phase II and III trials (only 3 randomized due to inclusion criteria of the meta-analysis) comprising 2,810 patients reported an RR of 22.6% and a disease control rate [DCR; CR + PR + stable disease (SD)] of 57.3% (Eckel and Schmid, 2007). The best RRs were seen in regimens combining gemcitabine with a platinum agent and shorter OS was noted specifically for GBC. Furthermore, a retrospective meta-analysis of 413 patients with aBTC supported gemcitabine as the backbone for cytotoxic treatment (Yonemoto *et al.*, 2007).

Recent randomized trials for aBTC set the current standard, based on the ABC-01 (Advanced Biliary Cancer) and ABC-02 trials that showed a significant advantage to doublet chemotherapy with cisplatin and gemcitabine in comparison to gemcitabine alone (Valle *et al.*, 2010; 2009). In the phase III trial, mOS of 11.7 months in the combination arm vs. 8.1 months in the gemcitabine alone arm was seen (HR 0.64, 95% CI 0.52-0.80; $p<0.001$) and mPFS was 8.0 months vs. 5.0 months (HR 0.63, 95% CI 0.51-0.77; $p<0.001$). The combination regimen was relatively well tolerated with similar toxicity to gemcitabine alone, and treatment was continued for up to 24 weeks (8 three-week cycles). A Japanese phase II trial of gemcitabine with cisplatin vs. gemcitabine alone found very similar results with mOS of 11.2 months in the combination arm (Okusaka *et al.*, 2010).

Based on these randomized trials, currently chemotherapy combining gemcitabine with a platinum agent is the recommended first-line treatment regimen for aBTC, outside of a clinical trial (Valle, 2010).

With respect to second-line palliative therapy, a recent retrospective analysis of 395 patients over 20 years at Princess Margaret Hospital in Toronto showed that after first-line chemotherapy, 25% of patients received a second-line regimen, and only 6% a third-line regimen (Walter *et al.*, 2012). Objective RRs and SD with second-line chemotherapy was 10% and 35%, respectively; mPFS and mOS measured from the initiation of second-line chemotherapy was 2.8 and 8 months, respec-

Table 1. First and Second Line Chemotherapy Phase II/III Trials in Advanced Biliary Tract Cancers.

Chemotherapy	Trial Characteristics				Response Rate (%)				Clinical Outcomes (months)			References
	Phase	N	R	Line	Regimen	CR	PR	SD	Regimen (comments)	PFS TTP	OS	
First Line Therapy												
Gem/CDDP vs. Gem	II	86	Y	1st	Gem/Gem/CDDP	0/0	22.6/27.8	35.5/47.2	Gem/Gem/CDDP	4/8	NR/NR	Valle <i>et al.</i> , 2009
Gem/CDDP vs. Gem	III	410	Y	1st	Gem/Gem/CDDP	0.7/0.6	14.8/25.5	56.3/55.3	Gem/Gem/CDDP	5/8	8.1/11.7	Valle <i>et al.</i> , 2010
Gem/CDDP vs. Gem	II	83	Y	1st	Gem/Gem/CDDP	0/0	11.9/19.5	38.8/48.8	Gem/Gem/CDDP	3.7/5.8	7.7/11.2	Okusaka <i>et al.</i> , 2010
MMC/Gem vs. MMC/Cape	II	51	Y	1st	MMC/Gem/MMC/Cape	0/0	20/31	36/34	MMC/Gem/MMC/Cape	4.2/5.3	6.7/9.25	Kornek <i>et al.</i> , 2004
5-FU vs. 5-FU/FA/CDDP	II	58	Y	1st	5-FU/5-FU/FA/CDDP	0/4	7/15	46/44	5-FU/5-FU/FA/CDDP	3.3/3.3	5/8	Ducréux <i>et al.</i> , 2005
FELV vs. ECF	III	54	Y	1st	FELV/ECF	0/3.8	15/15.4	45/46.2	FELV/ECF	7.3/5.2	12/9	Rao <i>et al.</i> , 2005
GEMOX	II	56	N	1st/2nd	Good PS/Poor PS	0/4	33/17	24/30	Good PS/Poor PS	5.7/3.9	15.4/7.6	Andre <i>et al.</i> , 2004
GEMOX	II	31	N	1st		0	26	45		6.4	11	Harder <i>et al.</i> , 2006
GEMOX	II	53	N	1st		1.9	17	51		4.8	8.3	Jang <i>et al.</i> , 2010
5-FU/FA	II	28	N	1st		7.1	25	21		NR	6	Choi <i>et al.</i> , 2000
5-FU/CDDP	II	42	N	Any line		0	43	31		3.5	7.5	Kobayashi <i>et al.</i> , 2006
FOLFOX	II	49	N	1st	CR+PR reported together	NR	16.3	45.4		3.8	10.8	Lee <i>et al.</i> , 2011
Cape/Ox	II	65	N	1st	GBC (n=27)/EHCC (n=20)/IHCC (n=18)	4/5/0	26/20/0	33/70/33	GBC/EHCC/IHCC	4.7/11.3/2.2	8/16.6/5.2	Nehls <i>et al.</i> , 2008
Cape/CDDP	II	32	N	1st		0	40.6	9.4		3.5	12.4	Hong <i>et al.</i> , 2007
Cape/CDDP	II	42	N	1st		2.3	19	28.6		3.7	9.1	Kim <i>et al.</i> , 2003
Gemcitabine	II	32	N	1st		0	21.8	43.7		3.7	9.1	Penz <i>et al.</i> , 2001
Gemcitabine	II	18	N	1st		0	22	28		3.4	8	Gebbia <i>et al.</i> , 2001
GemCape	II	75	N	1st		4	25	48		6.2	12.7	Riechelmann <i>et al.</i> , 2005
GemCape	II	45	N	1st		4	27	42		7	14	Knox <i>et al.</i> , 2005
Gem/5-FU	II	42	N	1st		0	12	NR		4.6	9.7	Alberts <i>et al.</i> , 2005
Irinotecan/5-FU	II	30	N	1st		0	10	10	IHCC/GBC	2.8/5.3	5.5/9.1	Feisthammel <i>et al.</i> , 2007
Irinotecan/Gem	II	14	N	1st		0	14	43		NR	NR	Bhargava <i>et al.</i> , 2003
Gemcitabine/S-1 (GS) vs. S-1 alone (S)	II	101	Y	1st	GS/S	NR/RR	NR/RR	NR/RR	GS/S	7.1/4.2	12.5/9	Ueno <i>et al.</i> , 2012
5-FU/FA/Oxaliplatin/Irinotecan	II	53	N	1st	Korean/Open	NA	NA	NA		NA	NA	NCT01494363
Second Line Therapy												
Gemcitabine after 5-FU based CTX	II	29	N	2nd		0	7	21		1.6	4.1	Oh <i>et al.</i> , 2011
5-FU/Doxo/MMC after Gem based CTX	II	16	N	2nd		0	13	26		2.3	6.7	Lee <i>et al.</i> , 2009
S-1 after Gem-based CTX	II	41	N	2nd		0	7.5	55		2.5	7.5	Suzuki <i>et al.</i> , 2010
S-1 after Gem-based CTX	II	22	N	2nd		0	23	27		5.4	13.5	Sasaki <i>et al.</i> , 2012

Abbreviations: Gem, gemcitabine; CDDP, cisplatin; MMC, mitomycin; Cape, capecitabine; 5-FU, 5-fluorouracil; FA, folic acid; OX, oxaliplatin; Doxo, doxorubicin; FOLFOX, 5-FU and Oxaliplatin; GS, gemcitabine with S-1; S, S-1; CTX, chemotherapy; OS, overall survival; PFS, progression-free survival; TTP, time to progression; CR, complete response; N, total number of patients in trial that were evaluable; R, randomized trial or not; Phase, phase I, II or III trial; Line, line of treatment (1st or 2nd or any); PR, partial response; RR, response rate; SD, stable disease; NR, not reported; NA, not applicable; S-1, tegafur/gimeracil/oteracil potassium.

tively. Few prospective trials of second-line chemotherapy or beyond for aBTC have been conducted (Table 1). In general, the average DCR was 30%, mPFS approximately 2 months, and mOS 4-6 months (Lee *et al.*, 2009; Oh *et al.*, 2011; Sasaki *et al.*, 2012; Suzuki *et al.*, 2010). The pivotal ABC-02 trial (Valle *et al.*, 2010) reported that only 17% of patients received second-line chemotherapy, although in the Japanese trial of gemcitabine with cisplatin, that rate was 70% (Okusaka *et al.*, 2010).

Current unanswered questions surrounding cytotoxic agents include exploring the triplet FOLFIRINOX (5-FU/ leucovorin, oxaliplatin, and irinotecan) for aBTC (open in Korea; NCT01494363); a genotype directed irinotecan dosing study of FOLFIRINOX in untreated patients with advanced gastrointestinal malignancies (including aBTC) is enrolling at the University of Chicago (NCT01592383). Other questions include maintenance chemotherapy in those with disease control after the initial 4-6 months of treatment and addressing issues surrounding the optimal use of combination chemotherapy with targeted agents.

Towards Personalized Therapy for Advanced Biliary Tract Cancers: Molecular Classification and Novel Targeted Agents in Clinical Trials

BTC arise from the epithelial lining of the biliary system which undergoes a sequence of steps including dysplasia, hyperplasia, carcinoma *in situ*, and finally invasive carcinoma. A number of molecular aberrations driving this progression have been identified and will be discussed below (Faris and Zhu, 2012; Furuse and Okusaka, 2011; Zhu and Hezel, 2011). Tables 2 and 3 summarize clinical trials using novel molecular targeting agents. Figures 2 and 3 schematically summarize our current understanding of important molecular pathways, targets, and their inhibitors in aBTC.

Epidermal growth factor receptor/ErbB1

EGFR is expressed in the majority (38-100%) of BTC (Pignochino *et al.*, 2010). Overexpression has been described in both GBC and EHCC (Lee and Pirdas, 1995). Sustained EGFR activation has been reported in cholangiocarcinoma cell lines, and EGFR tyrosine kinase inhibitors (TKIs) lead to decreased proliferation (Yoon *et al.*, 2004). In another report, overexpression of EGFR was only seen in 8.1% of cases, and 77% of those had EGFR gene amplification (Nakazawa *et al.*, 2005). It was noted that EGFR was overexpressed in 27.4% of IHCC and 19.2 % of EHCC, and a survival benefit was observed in EGFR-positive tumors (Yoshikawa *et al.*, 2008). EGFR mutations/exon 19

deletions have been described; in one report, 6 of 40 (15%) analyzed BTC harbored a somatic mutation in the EGFR tyrosine kinase domain and in another study 3 of 22 (13.6%) patients had EGFR mutations; both reports included previously known "hot-spots" and some novel mutations (Gwak *et al.*, 2005; Leone *et al.*, 2006). Interestingly, bile acids have been shown to activate EGFR (Werneburg *et al.*, 2003). EGFR overexpression, therefore, is frequent across BTC subtypes. EGFR mutations/amplifications and EGFR pathway activation collectively provide rationale for EGFR targeted inhibition strategies (Thomas, 2007). In addition, KRAS mutations, which in colon cancer have been shown to predict lack of response to EGFR inhibitors, are also noted in BTC, although the rate varies widely depending on the series, anywhere from 6% to 50%, and has in some studies been associated with worse clinical outcome (Malats *et al.*, 1995; Pignochino *et al.*, 2010).

Based on this background, a single arm phase II trial of 42 patients, of whom over 57% had prior systemic therapy, using erlotinib (an oral EGFR/HER1 TKI) produced 3 PRs and 17 SDs with a 17% 6-month PFS rate and a 31% 12-month OS (Philip *et al.*, 2006). Interestingly, out of the 36 patients examined for EGFR/HER1 status, seven (19%) lacked EGFR/HER1 expression of which none were progression free at 24 weeks, whereas 28% of the EGFR/HER1-positive patients were progression free at 6 months. Overall, time-to-progression (TTP) and mOS were 2.6 and 7.5 months, respectively. A recent open-label, non-placebo controlled phase III trial of 133 patients with aBTC (unselected for EGFR expression, mutation, or amplification) treated with GEMOX with or without erlotinib showed a statistically improved objective response rate (PR + CR) in the combination arm (40 patients vs. 21 patients; p=0.005), but no difference in PFS [4.2 months GEMOX group vs. 5.8 months GEMOX plus erlotinib group; (HR 0.80, 95% CI 0.61 -1.03; p=0.087) or mOS of 9.5 months in the GEMOX group and 9.5 months in the GEMOX plus erlotinib group; (HR 0.93, 0.69-1.25; p=0.611)] (Lee *et al.*, 2012). Interestingly, subgroup analysis showed that in those patients with CC, the addition of erlotinib prolonged PFS from 3.0 months to 5.9 months (HR 0.73, 95% CI 0.53-1.00; p=0.049). EGFR overexpression was reported in 43% (12 of 48 patients) with 1 PR and 7 SDs. Of 60 patients from the investigational arm examined, 10% had KRAS mutation (one with a 7.2 month response and another with an 11.4 month response; one with SD for 4.9 months and one for 11 months, one progressed quickly after 1.5 months).

A number of trials examined the use of cetuximab, a

Table 2. Clinical Trials Incorporating Novel Molecularly Targeted Agents.

Drug	Trial Characteristics				Target	Response Rate (%)				Clinical Outcomes (months)			Reference
	Phase	N	R	Line		Regimen	CR	PR	SD	Regimen (comments)	PFS TTP	OS	
Erlotinib	II	42	N	1st/ 2nd	EGFR		0	8	43		2.6	7.5	Philip <i>et al.</i> , 2006
GEMOX +/- Erlotinib (E)	III	268	Y	1st	EGFR	GEMOX/E GEMOX	0 2	30 14	36 51	GEMOX/E GEMOX	5.8 4.2	9.5 9.5	Lee <i>et al.</i> , 2012
Cetuximab + GEMOX (after GEMOX)	II	9	N	2nd	EGFR		11	22	22	Low EGFR High EGFR	4 7	7 9	Paule <i>et al.</i> , 2007
Cetuximab + GEMOX	II	30	N	1st	EGFR		10	53	17	30% had 2nd curative resection PFS 21.2 m	8.8	15.2	Gruenbeger <i>et al.</i> , 2010
GEMOX + panitumumab (KRAS wt)	II	42	N	1st	EGFR		2	31	52		8.3	10	Jensen <i>et al.</i> , 2012
Cetuximab + GEMOX CR + PR reported as one	II	150	Y	1st	EGFR	GEMOX GEMOX/C	NR NR	29 23	48 64	GEMOX GEMOX/C	5.3 6	12.4 11	Malka <i>et al.</i> , 2012
Gemcitabine/ Irinotecan/ Panitumumab	II	21	N	NR	EGFR		14	29	48	No EGFR or BRAF mut. in 13 samples; 7 KRAS mut	NR	12.7	Sohal <i>et al.</i> , 2012
Lapatinib	II	17	N	1st/ 2nd	EGFR and Her2		0	0	26		1.8	5.2	Ramanathan <i>et al.</i> , 2009
Sorafenib + Erlotinib	II	30	N	1st	EGFR and VEGF		0	7	27	59% with grade 3/4 toxicity	2	6	El-Khoueiry <i>et al.</i> , 2012b
Sorafenib	II	31	N	1st	VEGF		0	0	31		3	9	El-Khoueiry <i>et al.</i> , 2012a
Sorafenib	II	46	N	1st/ 2nd	VEGF		0	2.2	30		2.3	4.4	Bengala <i>et al.</i> , 2010
Gemcitabine +/- Sorafenib	II	62	Y	1st	VEGF		0	7	63		2.9	9.4	Moehler <i>et al.</i> , 2011
Sunitinib	II	56	N	2nd	VEGF		0	9	41		1.7	4.8	Yi <i>et al.</i> , 2012
GEMOX + Bevacizumab (mostly 1st line)	II	35	N	1st/ 2nd	VEGF		0	40	29		7	12.7	Zhu <i>et al.</i> , 2010
Bevacizumab + Erlotinib	II	49	N	1st	EGFR and VEGF		0	12	51		4.4	9.9	Lubner <i>et al.</i> , 2010
MEK162 (ARRY-438162)	I	26	N	1st/ 2nd	MEK		4	4	42		NR	NR	Finn <i>et al.</i> , 2012
Selumetinib	II	28	N	1st or 2nd	MEK		0	12	68		3.7	9.8	Bekaii-Saab <i>et al.</i> , 2011
Bortezomib	II	20	N	2nd/ 3rd	NF-κB		0	5	45		1.6	9.5	Costello <i>et al.</i> , 2009

Abbreviations: GEMOX, gemcitabine and oxaliplatin; E, erlotinib; C, cetuximab; wt, wild-type; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; MEK, mitogen-activated ERK kinase; N, total number of patients in trial that were evaluable; R, randomized trial or not; Phase, phase I, II or III trial; Line, line of treatment (1st or 2nd or any); OS, overall survival; PFS, progression-free survival; TTP, time to progression; NR, not reported; d, days; exp, expression; mut, mutations. * Unless otherwise specified numbers refer to months.

Table 3. Ongoing Trials Incorporating Novel Molecularly Targeted Agents.

Drug	Trial Characteristics			Target	Primary Objective	Country	Accrual Goal	Open/Closed	Identifier
	Phase	R	Line						
GEMOX +/- cetuximab	II	Y	1st	EGFR	Response Rate	Taiwan	120	Open	NCT01267344
GEMOX +/- erlotinib	III	Y	1st	EGFR	RP2D	Korea	180	Closed	NCT01149122
GEMOX +/- erlotinib	I	N	1st	EGFR	Response Rate	USA	22	Open	NCT00987766
GEMOX +/- panitumumab in KRAS/BRAF wt	II	N	1st	EGFR	Response Rate	USA	30	Open	NCT01308840
GEMOX/capecitabine +/- panitumumab (based on KRAS status)	II	Y	Any line	EGFR	PFS	Denmark	70	Open	NCT00779454
GEMOX +/- panitumumab	II	Y	1st	EGFR	PFS	Italy	18	Open	NCT01389414
Gemcitabine/irinotecan + panitumumab	II	N	1st	EGFR	PFS	USA	45	Open	NCT00948935
Gemcitabine/cisplatin +/- panitumumab in KRAS wt	II	Y	1st	EGFR	PFS	Germany	92	Open	NCT00948935
mFOLFOX6 + bevacizumab	II	N	1st	VEGF	PFS	USA	7	Closed	NCT00881504
Gemcitabine/capecitabine + bevacizumab	II	N	1st	VEGF	PFS	USA	50	Open	NCT01007552
mFOLFOX + cediranib	II	N	1st	VEGF	Response Rate	USA	25	Open	NCT01229111
Gemcitabine/cisplatin +/- cediranib	II/III	Y	1st	VEGF	PFS	UK	136	Open	NCT00939848
GEMOX + sorafenib	I/II	N	Any/ 1st	VEGF	PFS and RPTD	USA	58	Open	NCT00955721
Gemcitabine +/- vandetanib or vandetanib alone	II	Y	1st	VEGF	PFS	Italy	174	Open	NCT00753675
Gemcitabine/capecitabine + vandetanib	I	N	Any	VEGF	MTD	USA	28	Unknown	NCT00551096
Everolimus	II	N	1st	mTOR	Tumor Control	Australia	27	Unknown	NCT00973713
AZD6244	II	N	1st or 2nd	MEK	Response Rate	USA	35	Unknown	NCT00553332
GSK1120212 followed by GSK1120212 with gemcitabine	I	N	Any	MEK	Safety	Japan	19	Open	NCT01324258
Gemcitabine/cisplatin + selumetinib	I/II	N	Any	MEK	Safety/RPTD	UK	18	Pending	NCT01242605
MK2206	II	N	2nd	AKT	Response Rate	USA	35	Open	NCT01425879
Gemcitabine/cisplatin + veliparib	I	N	1st	PARP1/2	MTD	USA	44	Open	NCT01282333
Crizotinib (PF-02341066)	I	N	Any	MET/ROS /ALK	Safety/MTD	USA	475	Open	NCT00585195
GDC-0980	I	N	Any	PI3K/ mTOR	Safety/MTD	USA	105	Open	NCT00854152

Abbreviations: GEMOX, gemcitabine and oxaliplatin; wt, wild-type; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; MEK, mitogen-activated ERK kinase; mTOR, mammalian target of rapamycin; PARP1/2, Poly (ADP-ribose) polymerase; PFS, progression-free survival; MTD, maximally tolerated dose; RP2D, recommended phase II dose; NR, not reported; R, randomized trial or not; Phase, phase I, II or III trial; Line, line of treatment (1st or 2nd or any); Identifier, ClinicalTrials.gov number. * Unless otherwise specified numbers refer to months.

chimeric anti-EGFR monoclonal antibody, in aBTC. A case report demonstrated the feasibility of combining gemcitabine with cetuximab (Sprinzl *et al.*, 2006). This led to a 9-patient trial of cetuximab with GEMOX in aBTC patients who had progressed on GEMOX, showing 100% EGFR expression by IHC, but no EGFR gene amplification, and mPFS and mOS of 4 and 7 months, respectively, with a response rate of 33% -- raising the possibility of cetuximab overcoming resistance to previously used cytotoxic therapy (Paule *et al.*, 2007).

A single arm phase II study of cetuximab, gemcitabine, and oxaliplatin in 30 aBTC patients showed a 63% response rate, mPFS of 8.8 months, and mOS of 15.2 months, with 30% being able to undergo curative-intent resection after chemotherapy (Gruenberger *et al.*, 2010). *KRAS* mutations were again reported in 10%. There were 2 PRs and one SD -- all, intriguingly, in *KRAS* mutant patients. This is somewhat unexpected given the negative predictive nature of *KRAS* mutation in colorectal cancer to anti-EGFR therapy. Also, all patients with a grade 2 or 3 skin rash had a PR or CR,

and toxicity was deemed acceptable. Finally, the open-label, 150 patient (79% CC), randomized phase II BINGO trial of GEMOX with or without cetuximab was recently presented (Malka *et al.*, 2012). In the 131 evaluable patients, the overall RR was higher, 29%, in the GEMOX only arm compared to 23% in the investigational arm, yet the disease control rate was 77% with GEMOX and 87% in the combination arm. The 4-month PFS rate, the trial primary endpoint, was 53% with GEMOX compared to 63% in the combination arm. However, mOS was 12.4 months vs. 11 months in favor of GEMOX alone.

A recent study of gemcitabine, irinotecan, and panitumumab, a fully humanized monoclonal antibody to EGFR, in aBTC showed a 90% DCR and mOS of 12.7 months in 21 evaluable patients (Sohal *et al.*, 2012). There were 53% with *KRAS* mutations and no *EGFR* or *BRAF* mutations noted. Finally, a trial of GEMOX with panitumumab in 46 *KRAS* wild-type patients showed mPFS of 8.3 months and mOS of 10 months; it was reported that 60-90% of BTC were *KRAS* wild-type, a

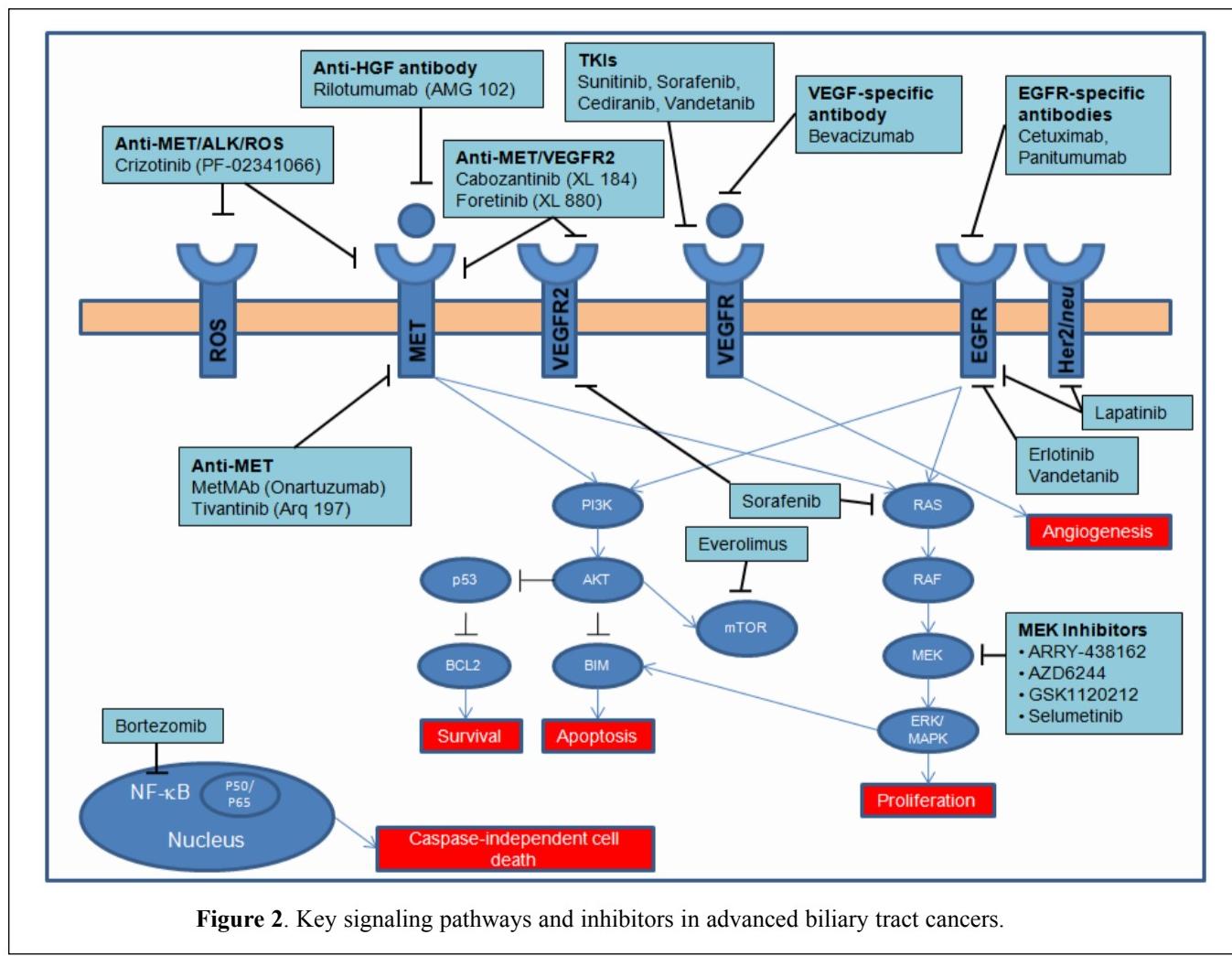


Figure 2. Key signaling pathways and inhibitors in advanced biliary tract cancers.

number significantly higher than reported previously (Jensen *et al.*, 2012).

Clearly, based on the above trials, in an unselected aBTC patient population, the addition of anti-EGFR agents does not result in obvious clinical improvement over a standard gemcitabine/platinum doublet. Currently there are at least 8 ongoing trials of EGFR-targeted agents in various stages for aBTC, and in particular several trials which are specifically enriching for KRAS wild-type patients (Table 3).

HER2/neu (ErbB2)

Overexpression of HER2/neu has generally been seen in anywhere from 1-16% of BTC, mostly in GBC, although it has been noted in 30% of IHCC in one series (Nakazawa *et al.*, 2005; Pignochino *et al.*, 2010; Sirica, 2008; Yoshikawa *et al.*, 2008). In a recent study, HER2 upregulation was associated with a poor prognosis in BTC patients (Andersen *et al.*, 2012). Another recent study evaluating single agent lapatinib, a dual oral EGFR and HER2/neu kinase inhibitor, did not show single agent activity in aBTC for 1st or 2nd line treatment (Ramanathan *et al.*, 2009). There were no objective responses, 26% DCR, and mOS of 5.2 months. The trial did not enrich for a HER2/neu or EGFR popula-

tions, so it remains unclear if there is differential benefit in this sub-population.

Vascular endothelial growth factor (VEGF)

VEGF, a key factor of angiogenesis facilitating tumor growth and metastasis, is expressed in 30-50% of BTC; VEGF levels may be associated with worse prognosis in EHCC (Giatromanolaki *et al.*, 2003; Hida *et al.*, 1999; Quan *et al.*, 2001; Yoshikawa *et al.*, 2008). A trial of GEMOX with bevacizumab, a recombinant and fully humanized monoclonal antibody towards VEGF, was examined in a phase II trial of 35 patients with aBTC (Furuse, 2010; Zhu *et al.*, 2010b). An RR of 40% and mPFS/OS of 7.0/12.7 months compared favorably with historic controls.

Three trials have examined sorafenib, an oral non-specific TKI targeting the Ras-Raf-Erk pathway as well as VEGFR2/3 and PDGFR. The SWOG 0514 trial evaluated sorafenib alone for first line treatment in 31 evaluable patients and observed no responses, 31% SD rate, and mPFS/OS of 3 and 9 months (El-Khoueiry *et al.*, 2012a). A randomized phase II trial examined gemcitabine alone or with sorafenib for first-line therapy and in 62 patients found a 7% PR rate, 63% SD rate, and mPFS/OS of 2.9 and 9.5 months (Moehler *et al.*, 2011).

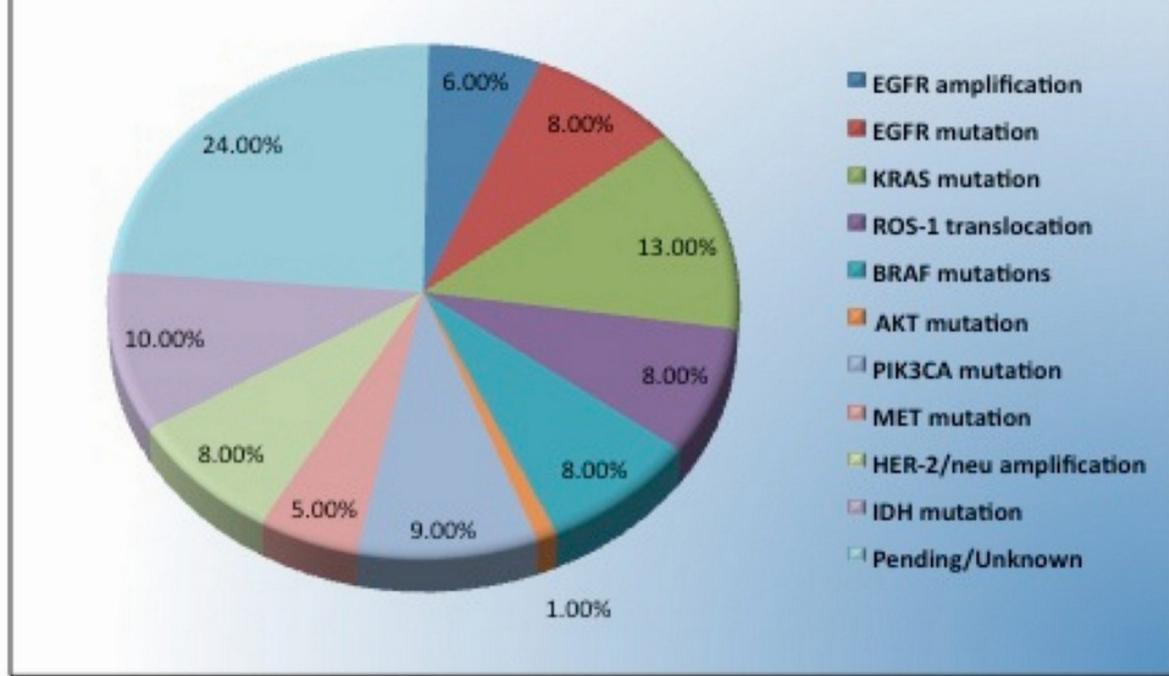


Figure 3. Current overall estimated frequency of genetic aberrations in BTC with potential as “actionable” targets. Relative frequencies vary widely based on BTC sub-type and study. Estimates are based on small sample cohorts. One or more aberrations may occur within an individual tumor but are not considered in this figure.

Another phase II trial of 46 patients, of whom 56% had prior chemotherapy, were treated with single agent sorafenib and found to have a 2% PR and a 30% SD rate with PFS and OS of 2.3 and 4.4 months (Bengala *et al.*, 2010). In second-line therapy, single agent sunitinib (another multi-kinase inhibitor of VEGFR, PDGFR, and others) in 56 patients was reported to have an RR of 8.9% (5 PRs), TTP of 1.7 months, and mOS of 4.8 months (Yi *et al.*, 2012).

Currently there are at least seven ongoing trials of antiangiogenic agents including bevacizumab, cediranib (VEGFR2/3 TKI), sorafenib, and vandetanib -- a novel VEGFR, EGFR, and RET-tyrosine kinase inhibitor which has been shown to have preclinical activity in cholangiocarcinoma cell lines (Yoshikawa *et al.*, 2009) (Table 3). We await the results of these trials and urge parallel molecular tissue correlative analyses attempting to discover positive and negative predictive biomarkers of relevant clinical outcomes.

Combinatorial use of EGFR and VEGF targeted therapy for aBTC

A recent case report of dual therapy with panitumumab and bevacizumab in a patient with widely metastatic GBC unfit for any cytotoxic therapy demonstrated a significant PR and improvement in performance status for 7 months (Riley and Carlsson, 2011). A phase II study of 49 evaluable patients with chemotherapy-naïve aBTC investigated EGFR/VEGF inhibition with erlotinib and bevacizumab (Lubner *et al.*, 2010). Six confirmed PRs were noted with a median duration of response being 8.4 months in those patients. Overall mTTP was 4.4 months and mOS was 9.9 months. Exploratory analysis of EGFR mutational status showed that those with EGFR truncation variant III or those with KRAS mutation suggested a less likely response to erlotinib; serum VEGF expression was not noted to change from baseline between responders and nonresponders. Recently, the SWOG 0941 trial enrolled 30 evaluable patients to receive first-line therapy with daily sorafenib and erlotinib with primary endpoint to improve PFS from 4 to 8 months (El-Khoueiry *et al.*, 2012b). Two patients had a PR and 8 had SD as their best response, but there were 3 deaths while on study with one possibly related to treatment. The mPFS/OS was 2 and 6 months and the trial was stopped early due to a weak efficacy signal. Further studies are required to assess whether there may be benefit in certain subsets of patients.

MEK (mitogen-activated ERK kinase)

The RAS/RAF/MEK/ERK (MAPK) pathway is activat-

ed by numerous growth signals via their receptors, including EGFR, and is crucial in mediating uncontrolled growth and survival (De Luca *et al.*, 2012). A preclinical study in a murine orthotopic model using a human gallbladder cell line harboring KRAS mutation, demonstrated constitutive MAPK activation and progression of GBC; MEK inhibitors, U0126 and PD98059, significantly prolonged survival of mice compared to untreated controls (Horiuchi *et al.*, 2003; 2004). In the largest MEK inhibitor clinical trial to date for aBTC, a phase II study of selumetinib, an oral MEK1/2 inhibitor, was conducted in 28 patients of whom 39% had prior therapy and 61% had IHCC (Bekaii-Saab *et al.*, 2011). The trial was designed to determine the safety and efficacy of selumetinib with the primary endpoint of response rate. Levels of phosphorylated ERK (pERK) and pAkt were measured via immunohistochemistry (IHC) and tumors were assessed for BRAF and KRAS mutations. They reported a 12% (3 patients) PR and 68% (17 patients) DCR and 14 of 17 with SD for over 4 months and 3 patients experiencing SD for over 1 year. Additionally, 52% had at least partial tumor regression during the study. The mPFS/OS were 3.7 and 9.8 months with manageable toxicities. They observed that lack of pERK staining was associated with a lack of response in their pre-planned exploratory studies; no BRAF mutations and two KRAS mutations were noted. Recently, Finn *et al.* (2012) reported on phase I expansion cohort of aBTC patients receiving single agent MEK1/2 inhibitor, ARRY-438162. Twenty-eight patients, either on first- or second-line treatment (43% with past chemotherapy, primarily gemcitabine based) were enrolled and tumor samples were assessed for KRAS and BRAF mutation status. There were 6 mutations noted in 23 assessed tumors (1 MET, 1 PIK3CA, 2 KRAS, 2 PTEN null) with grade 3 or 4 adverse events being anasarca, electrolyte abnormalities, GI bleeding, and mucositis (4 patients) with 3 patients stopping for adverse events. Of the 26 patients evaluable for response, there was 1 CR, 1 PR, and 11 SD -- both responders had EHCC and did not harbor any mutations. This is somewhat unexpected since preclinical models seem to suggest that MEK inhibition would most benefit those tumors with upstream KRAS mutation and constitutive activation. Regardless, these results are promising and currently there are 4 trials underway addressing the utility of various MEK inhibitors, including in combination with gemcitabine and cisplatin (Table 3).

NF-κB

Inhibition of the proteasome, an intracellular machinery responsible for protein degradation, can prevent the clearance of pro-apoptotic factors, thus halting neoplas-

tic cell growth and sensitizing neoplastic cells to further chemotherapy (Wiedmann and Mossner, 2010). Bortezomib, a proteasome inhibitor, mechanistically inhibits, in part, NF- κ B, a key transcriptional factor involved in the regulation of apoptosis (Voorhees *et al.*, 2003). Bortezomib was observed to inhibit CC cells *in vitro* with limited toxicity in normal rat cholangiocytes (Baradari *et al.*, 2007; Ustundag *et al.*, 2007). Based on these data, a single-arm phase II study was conducted with 20 patients having chemorefractory aBTC (1-2 prior regimens) treated with single agent bortezomib (Costello *et al.*, 2009). Unfortunately, the PR rate was 5% with 45% SD and PFS of 1.6 months and mOS of 9.5 months. The trial was halted as it did not meet its primary endpoint of greater than 5% response rate. There currently are no known ongoing trials with bortezomib for aBTC.

Future Directions and Novel Targets for Molecularly Designed Therapy

Recently, due to rapid technological advances (Stricker *et al.*, 2011) our ability to molecularly profile cancers' genomes, transcriptomes, and proteomes (and more) has revealed a number of other frequent molecular aberrations in BTC, both enhancing our understanding of the biology of the cancer and elucidating a number of actionable targets with available drugs. These include the MET receptor tyrosine kinase, the PIK3CA/AKT/mTOR pathway, ROS-1 translocations, BRAF mutations, as well as several emerging targets such as IDH1. It is becoming increasingly clear that BTC, as is observed in most if not all malignancies, are quite heterogeneous in their molecular biology, resulting in smaller and smaller subsets of classification within the traditional anatomical categories, which will potentially require an individual tumor profiling and personalized treatment plan.

MET

The *MET* gene, initially discovered in 1984, is a receptor tyrosine kinase with hepatocyte growth factor (HGF) as its sole ligand (Cooper *et al.*, 1984). Upon activation, it initiates a downstream intracellular cascade involved in tumor growth, cellular invasion, angiogenesis, and development of metastasis. A strong preclinical rationale has rapidly accelerated to clinical trials evaluating MET inhibitors in various cancers (Comoglio *et al.*, 2008), some with very encouraging results (Catenacci *et al.*, 2011b; Feng and Ma, 2011). MET is overexpressed in approximately 20-70% of BTC, predominantly highest observed in EHCC and IHCC (Aishima *et al.*, 2002; Nakazawa *et al.*, 2005; Socoteanu *et al.*, 2008; Terada *et al.*, 1998). A preclinical model of BTC activation of MET via HGF led to MEK1/2 and MAPK activation, resulting in CC cell invasion, a phenomenon abrogated by MET siRNA as well as exposure to a MEK inhibitor (Leelawat *et al.*, 2006). A recent study of 111 patients with IHCC and 136 patients with EHCC, all surgically resected, examined the prognostic role of MET (Miyamoto *et al.*, 2011). MET expression was detected in 45% of IHCC and 68.4% of EHCC with MET_{high} (2+ or higher by IHC) in 11.7% of IHCC and 16.2% of EHCC. MET_{high} expression was negatively correlated with 5-year OS in IHCC patients (5-year OS 41.1% in MET_{low} vs. 15.4% in MET_{high}), a signal observed in other malignancies (Catenacci *et al.*, 2011a; Graziano *et al.*, 2011). Also, MET expression was correlated with EGFR overexpression. There is strong rationale for evaluation of MET inhibitors for BTC, either alone or in combination with cytotoxic or other biologic agents, in particular EGFR inhibitors, as recent reports suggest in lung cancers (Engelman *et al.*, 2007; Sequist *et al.*, 2011). Later, we describe crizotinib, a multi-targeted TKI for MET and ROS-1 currently in phase I trials, that is being evaluated in BTC patients at the University of Chicago.

PIK3CA/PTEN/AKT/mTOR (mammalian target of rapamycin)

PIK3CA is a lipid kinase involved in the activation of downstream AKT, resulting in cell proliferation and survival (Samuels and Waldman, 2010). In BTC, 1 *PIK3CA* mutation in 11 IHCC and 1 in 23 GBCs was reported (Riener *et al.*, 2008). A study of 34 Chinese patients with CC (type not specified) analyzed tissue for hotspot mutations of *KRAS*, *BRAF*, and *PIK3CA* genes; somatic *KRAS* mutations were seen in 38.2% in this series (see discussion above), while *PIK3CA* was reported in 32.4% and *BRAF* in 0% of patients (Xu *et al.*, 2011). Another series of 77 patients with BTC detailed activating *PIK3CA* mutations in 12.5% of GBC only (Deshpande *et al.*, 2011). In another Chinese population of 34 patients, it was described that 11 (32.4%) tumors had a *PIK3CA* mutation (Xu *et al.*, 2011). In IHCC, in a cohort of 62 patients, 40.7% were moderately positive and 22% were strongly positive for pAKT by IHC (Schmitz *et al.*, 2007). Two clinical trials specific to BTC are currently targeting this pathway, one examining single agent everolimus in Australia, and a second, MK2206, an AKT inhibitor, in the U.S. for second-line use (NCT00973713; NCT01425879) (Table 3). Also, a phase I clinical trial (NCT00854152) at the University of Chicago is enriching for solid tumors with a *PIK3CA* mutation, including aBTC.

ROS-1

The proto-oncogene tyrosine kinase, ROS, is an enzyme over-expressed in many tumor types, and is closely related to ALK kinase and the insulin receptor family, and is involved in proliferation and differentiation (Birchmeier *et al.*, 1987). Elevated ROS expression has been noted in lung cancer and gliomas, and importantly, *ROS-1* translocations and consequent fusion proteins have been reported in lung cancer and with sensitivity to MET/ALK/ROS-1 inhibitor, crizotinib (Bergeron *et al.*, 2012). Previously, *ROS-1* translocation was observed in 2 of 23 (8.7%) cholangiocarcinoma patients (Gu *et al.*, 2011). As a result, at the University of Chicago, BTC patients are tested for the *ROS-1* translocation by FISH (fluorescence in situ hybridization) and, if present, are eligible for a crizotinib clinical study (NCT00585195).

IDH

As mentioned, novel high-throughput molecular profiling of BTC is being conducted at multiple centers. A report of broad-based tumor genotyping of 87 BTC (25 GBC and 62 CC) unexpectedly found a number of *IDH1* mutations (in 23% of IHCC and 0% of EHCC/GBCs) that have not yet been fully elucidated (Borger *et al.*, 2012). The *IDH1* mutation was the most common mutation observed in IHCC in this study. Incidentally, the mutation rate for *KRAS* of ~20%, for *AKT* of ~1%, and for *PIK3CA* of ~12% was noted. These and other findings will continue to help define the distinct molecular subsets of BTC and provide novel targets to investigate for potential therapeutic benefit.

BRAF

Alluded to above, *BRAF* is a proto-oncogene within the MAPK signaling pathway and is involved in the pathogenesis of many human cancers (Davies *et al.*, 2002). *BRAF* mutation frequency varies widely from study to study, in some series not detected while in others reported in up to 33% in GBC (Deshpande *et al.*, 2011; Saetta *et al.*, 2004; Xu *et al.*, 2011). Recent improvements in survival with *BRAF* inhibitors in *BRAF* mutant melanoma provides rationale to evaluate this strategy in *BRAF* mutant BTC (Sosman *et al.*, 2012).

Other mutations/pathways involved in BTC

Many other candidate therapeutic targets have been identified in BTC. These include the Notch signaling pathway which has been shown to be aberrantly expressed in EHCC and GBC, with potential prognostic value (Yoon *et al.*, 2011), insulin growth factor receptor (IGFR) which is expressed in the majority of GBC (Kornprat *et al.*, 2006), and sonic hedgehog sig-

naling pathway whose proteins have been shown to be overexpressed in EHCC, the inhibition of which led to decrease in proliferation of EHCC cell lines (Kim *et al.*, 2012).

Conclusions

BTC are a heterogeneous group of diseases -- anatomically, histologically, and biologically (Hezel *et al.*, 2010). In general, due to vague symptoms, patients with these cancers present at late disease stages and overall survival is poor. Historically, cytotoxic agents for aBTC have had only mild to moderate activity, while the current standard-of-care of gemcitabine and cisplatin attains an mOS of approximately 1 year. Over the last years a number of molecular pathways have been implicated in the pathogenesis of BTC and recent technological advances have provided the capability to rapidly screen for a wide array of molecular aberrations, many of which are “actionable” putative therapeutic targets (Stricker *et al.*, 2011). Trials with specific agents towards these targets in BTC have to date not produced obvious success, in part due to the difficulty in conducting trials for this relatively rare malignancy, but also due to the anatomical and biological heterogeneity of the disease. As a result of this recognized inter-patient heterogeneity, a one-size-fits-all treatment approach with novel biologics has repeatedly failed across tumor types, while highly selective clinical trials enriching for “driver” aberrations (also referred to as oncogene addiction), paired with rational therapeutic agents selective for these drivers, have had better success. Novel actionable driver oncogenes are continuingly being identified as putative targets of BTC, including EGFR, VEGFR, MEK, MET, *ROS-1*, *IDH*, and others.

We are at the dawn of personalized cancer care for BTC. The relative rarity of this cancer has resulted in a lag in the advances in understanding of the underlying molecular biology as well as the introduction of novel therapeutic agents, compared to the more common malignancies. Enrollment in large cooperative group trials can overcome this issue and will enable the accrual of adequate numbers of patients to provide the desperately needed answers to carefully constructed questions. Clinical trial design will need to evolve to address our current understanding of the heterogeneity of cancer between and within patients, at diagnosis and through therapy. In the next years, the approach of personalized care by molecularly profiling tumors at diagnosis and then rationally combining standard chemotherapy and targeted agents will need to be tested for benefit with unprecedented ingenuity (Stricker *et al.*, 2011). The hope for improved outcomes for BTC lies in this personalized approach -- the future is now.

Disclosure

The authors report no conflicts of interest.

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